(43)

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Antisense Oligodeoxynucleotide Evidence That a Unique Osteoclastic Protein-Tyrosine Is Essential for Osteoclastic Resorption

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ABSTRACT

This study tested the hypothesis that a unique osteoclastic transmembrane protein tyrosine phosphatase (PTP-oc) is involved in osteoclastic resorption by determining whether suppression of PTP-oc expression with a specific phosphorothioated 20-mer PTP-oc antisense oligodeoxynucleotide (oligo) would inhibit basal, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]-stimulated, and PTH-stimulated osteoclastic resorption. Treatment of rabbit osteoclasts with 1 μM of the antisense oligo for up to 4 days showed a time-dependent reduction in PTP-oc protein level, indicating that this PTP-oc antisense oligo was effective. To assess the effect of PTP-oc antisense oligo on osteoclastic resorption, rabbit osteoclasts were pretreated for 3 days with 1 μM of the antisense, a scramble oligo, or vehicle,

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respectively, followed by a 3-day treatment with vehicle, 10 nM of $1,25(OH)_2D_3$, or 10 nM of parathyroid hormone (PTH). $1,25(OH)_2D_3$ and PTH each alone increased PTP-oc cellular level and stimulated resorptive activity of rabbit osteoclasts. The antisense oligo treatment, but not the scramble oligo, decreased the basal and the stimulated resorption activity and reduced the PTP-oc protein level. Treatment with the PTP-oc antisense oligo, but not the scramble oligo, also markedly increased the Y527 phosphorylation level of c-*src* in rabbit osteoclasts. In conclusion, these results provide the first antisense oligo evidence that PTP-oc plays an essential role in osteoclastic resorption.
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Key words: protein-tyrosine phosphatase; antisense oligodeoxynucleotides; osteoclasts; bone resorption(rabbit)

INTRODUCTION

PAST STUDIES with the op/op variant of murine osteopetrosis have indicated that the macrophage colony-stimulating factor (M-CSF) and activation of its receptor (c-*fms*), which contains an intrinsic protein tyrosine kinase (PTK) activity,((1)) are required for normal osteoclast formation.((2-4)) Studies in the c-*src* knockout mice also showed that abrogation of the c-*src* expression resulted in inactive osteoclasts and development of osteopetrosis.((5-7)) The c-*src* encodes a PTK. There is circumstantial evidence that the PTK activity of c-*src* may be involved in osteoclastic resorption. Accordingly, the PTK of c-*src* correlated significantly with the number of osteoclasts that were actively resorbing bones.((6)) Specific inhibitors of the c-*src* PTK activity have been shown to abolish completely the bone resorption activity of isolated osteoclasts on bone slices.((8)) The c-*src* PTK activity in osteoclasts is

stimulated by bone resorption activators such as parathyroid hormone (PTH) ((7)) and 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$],((9)) and is inhibited by resorption inhibitors such as calcitonin.((7)) In addition, recent studies showed that the PTK activity of c-*src* may play a role in regulating downstream signaling events, involving protein tyrosine phosphorylation, that are important to osteoclastic resorption.((10,11)) On the other hand, a recent study reported that transgenic expression of a K295M "dominant negative" c-*src* mutant (which is deficient in PTK activity) into c-*src* knockout mice partially rescued the osteopetrotic phenotype.((12)) This finding raises a question as to whether the PTK activity of c-*src* is absolutely essential for osteoclast activity. Nevertheless, there is an abundance of compelling evidence that protein tyrosine phosphorylation reactions mediated either by c-*fms* or c-*src* or by downstream PTKs is important, although they may not be absolutely required, for osteoclast maturation and manifestation of osteoclastic activity.

The role of protein tyrosine phosphatases (PTPs) in the regulation of osteoclast maturation and activation, contrary to that of PTKs, has been unclear. We have identified and cloned a novel transmembrane PTP, termed osteoclastic transmembrane PTP (PTP-oc), from a rabbit osteoclast complementary DNA (cDNA) library.((13)) This transmembrane PTP is structurally unique and is expressed predominantly in osteoclasts and precursors.((13)) This study sought to test the hypothesis that PTP-oc might play a key role in regulating osteoclastic resorption by testing whether suppression of the expression of PTP-oc (by a specific antisense oligodeoxynucleotide [oligo]) in isolated osteoclasts would reduce the basal or stimulated bone resorption activity assessed by the resorption pit formation assay.((14))

MATERIALS AND METHODS

Materials

Tissue culture supplies were obtained from Falcon (Oxnard, CA, USA); Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were products of Life Technologies (Grand Island, NY, USA). The enhanced chemiluminescence (ECL) detection kit was obtained from Amersham Corp. (Arlington Heights, IL, USA). Fuji X-ray films (Fuji Co., Ltd., Tokyo, Japan)

were purchased through local suppliers. Immobilon-P transfer membrane was a product of Millipore Corp. (Bedford, MA, USA). PTH(1-34) was obtained from Bachem (Torrance, CA, USA) and 1,25$(OH)_2D_3$ was a product of Biomol Biomolecules Research Laboratory (Plymouth Meeting, PA, USA). All other reagents were of reagent grade and were obtained from either Sigma Chemical Co. (St. Louis, MO, USA) or Fisher Chemicals Co., (Los Angeles, CA, USA).

Isolation of rabbit osteoclasts

Rabbit osteoclasts were isolated from long bones of 7- to 10-day-old New Zealand white rabbits (Irish Farm, Norco, CA, USA) according to Tezuka et al. ((15)) The isolated rabbit osteoclasts were >95% in purity and were used immediately for these studies.

Design of PTP-oc antisense oligo

The Primer 2 SES program (Scientific & Educational Software, Durham, NC, USA) was used to design the PTP-oc antisense oligo, which was a phosphorothioated 20-mer (5′-GsTsAsAsCsCsAsTsTsGsTsTsGsAsGsAsCsAsGsC-3′) corresponding to a region around the translation initiation site (-12 to +8) of the PTP-oc gene. The selected sequence was unique compared with known PTP sequences. A 20-mer phosphorothioated PTP-oc sense oligo corresponding to the same region (i.e., 5′-GsCsTsGsTsCsTsCsAsAsCsAsAsTsGsGsTsTsAsC-3′) and a scramble phosphorothioated 20-mer oligo (i.e., 5′-GsTsCsTsAsGsTsTsAsGsCsAsCsGsAsAsTsGsCsA-3′) also were synthesized as controls for comparison. Sequence homology search in the GenBank database with the Nucleotide Blast program revealed that the scramble oligo sequence did not match or was complimentary to the sequence of any known mammalian genes. These oligos were high-performance liquid chromatography (HPLC)-purified and were prepared by Genset Corp. (La Jolla, CA, USA).

Resorption pit formation assay

The bone resorption activity of isolated osteoclasts was measured with the resorption pit formation assay.((14)) Briefly, thin disc-shaped wafers ($7 \times 7 \times 0.5$

mm) of dentine were prepared with a low-speed diamond saw (Maruto, Kyoto, Japan). The dentine slices were then sterilized by sonication and stored in 95% ethanol at 4°C until use. Immediately before use, the dentine slices were rinsed with sterile phosphate-buffered saline and placed in 24-well culture plates. Then, isolated osteoclasts were plated on each dentine slice in DMEM supplemented with 10% fetal bovine serum for 3 days in the presence or absence of 1 μM of PTP-oc antisense or scramble oligo in humidified air (5% CO_2) at 37°C. After the pretreatment, the cells were cultured for 3 additional days in DMEM in the presence of each respective oligo, with or without 10^{-8} M of $1,25(OH)_2D_3$ or 10^{-8} M of PTH, to induce resorption pit formation. After the treatment, osteoclasts attached on the dentine slice were stained for tartrate-resistant acid phosphatase (TRAP) and the number of $TRAP^+$ multinucleated (more than two nuclei) osteoclasts on each slice was counted. After that, osteoclasts were removed from dentine slices by sonication and resorption lacunae were stained with acid hematoxylin. The number of resorptive lacunae was counted with the help of a computer-aided video camera under a light microscope and the lacunae area was quantified with a digitizing pad using a computer-assisted morphometric software program (OsteoMetrics, Decatur, GA, USA) on an image and process analysis system. The total measured area was 25.25 mm^2 (i.e., 5.05 mm $\times$ 5.05 mm) located at the center of the disc. The bone resorption activity per osteoclast (an index of mean osteoclast activity) was determined by dividing total resorption lacunae area by number of resorption pits.

PTP-oc Western immunoblot analysis

After appropriate treatments, cellular proteins of isolated rabbit osteoclasts were extracted with the radioimmunoprecipitation assay (RIPA) buffer (50 mM of Tris-HCl, pH 8.0, 1% [vol/vol] NP-40, 20 mM of EDTA, 0.1 mM of sodium orthovanadate, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 0.05 mM of leupeptin, 1 mM of phenylmethylsulfonyl fluoride [PMSF], and 10 μg/ml of aprotinin). The cell lysate was centrifuged at 10,000*g* for 15 minutes at 4°C and the protein concentration in each lysate was measured with the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). An equal amount of cellular protein (10-25 μg per sample) was analyzed with the Western immunoblot assay as previously described.((16)) The PTP-oc protein

band was identified with a specific anti-PTP-oc polyclonal antibody, generated in guinea pigs against the N-terminal fragment of PTP-oc-glutathione *S*-transferase (GST) fusion protein, followed by incubation with the goat-anti-guinea pig horseradish peroxidase (HRP)-conjugated secondary antibody and ECL. To normalize for protein loading, each blot, after PTP-oc Western blotting, was stripped and reblotted against an antiactin polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The relative amount of PTP-oc protein was quantified with laser densitometry and normalized against the actin protein level.

c-Src Y-527 phosphorylation level measurement

An equal amount of cellular protein of rabbit osteoclast lysates, after appropriate treatments, was separated on 10% SDS-polyacrylamide gel electrophoresis (PAGE). The relative Y-527 phosphorylation level of c-*src* was measured by Western immunoblot analysis using a phospho-specific polyclonal antibody against PY-527 of c-*src* (Biosource International, Camarillo, CA, USA). After Western blotting for c-*src* PY-527, the blot was stripped and reblotted against an antiactin polyclonal antibody and the relative amount of PY-527 was normalized against the actin protein level. To assess the effect of each oligo treatment on total c-*src* protein level in rabbit osteoclasts, each osteoclast lysate also was Western immunoblotted against a monoclonal (clone GD11) antibody against the c-*src* (Upstate Biotechnology, Lake Placid, NY, USA) in parallel analyses.

Statistical analysis

Results are shown as mean ± SEM of three to six replicates unless otherwise indicated. Statistical significance was determined with analysis of variance (ANOVA) followed by Tukey post hoc test or with two-tailed Student's *t*-test and the difference was considered significant when $p < 0.05$.

RESULTS

The isolated rabbit osteoclasts were functionally active, because they resorbed bone matrix in the resorption pit formation assay. Figure 1 shows that these osteoclasts were responsive to the stimulation by $1,25(OH)_2D_3$ (10

nM), PTH (10 nM), and prostaglandin E_2 (10 μM), respectively, as these agents stimulated the bone resorption activity of these rabbit osteoblasts by ~2-fold.

To ensure that the PTP-oc antisense oligo would effectively suppress the cellular PTP-oc protein expression in rabbit osteoclasts, we treated the osteoclasts (~500 cells/well) with 1 μM each of the phosphorothioated PTP-oc antisense or scramble oligo, respectively, for a period of 1-4 days. The vehicle-treated osteoclasts were included in parallel for comparison. Figure 2 shows that the cellular level of PTP-oc protein (normalized against actin) in osteoclasts treated with either the scramble oligo or the solvent vehicle did not change significantly during the 4-day treatment period. Treatment of rabbit osteoclasts with the PTP-oc sense oligo for the same time period also did not alter significantly the cellular PTP-oc protein level (normalized against actin; data not shown). In contrast, treatment of rabbit osteoclasts with the PTP-oc antisense oligo reduced significantly ($p < 0.001$, ANOVA) the cellular PTP-oc protein level (normalized against actin) in a time-dependent manner. Significant suppression of PTP-oc protein expression by the antisense oligo was seen after 3 days or 4 days of the treatment ($p < 0.05$ for 3 days and $p < 0.001$ for 4 days with the Tukey post hoc test), suggesting that 3-4 days of pretreatment with the antisense oligo would be sufficient to suppress cellular expression of PTP-oc in isolated rabbit osteoclasts.

We next assessed the effect of pretreatment with PTP-oc antisense or the scramble oligo on basal (vehicle-treated), $1,25(OH)_2D_3$-stimulated, and PTH-stimulated bone resorption activity of isolated rabbit osteoclasts in the resorption pit formation assay. Figure 3 illustrates that the size of resorption pits in PTH- and $1,25(OH)_2D_3$-treated osteoclasts was on the average bigger than that in vehicle-treated cells. More importantly, the average pit size in osteoclasts pretreated with PTP-oc antisense oligo, regardless of whether they were treated with or without PTH or $1,25(OH)_2D_3$, was much smaller than that in each corresponding control. On the other hand, the scramble oligo pretreatment did not alter the average pit size compared with respective control. The summarized results on total area of resorption pits (Fig. 4, top panel), number of pits (Fig. 4, middle panel), and area per pit (Fig. 4, bottom panel), respectively, for each treatment ($n = 6$) are shown in Fig. 4. In the

absence of any oligo pretreatment, PTH (10 nM) and $1,25(OH)_2D_3$ (10 nM) each increased significantly the total resorption pit area and area per pit ($p < 0.01$ for each) compared with vehicle-treated controls, confirming that these two agents increased the resorption activity of isolated rabbit osteoclasts. Importantly, the PTP-oc antisense oligo treatment reduced significantly ($p < 0.001$ for each) the basal (vehicle-treated) as well as PTH- and $1,25(OH)_2D_3$-stimulated bone resorption (reflected by total resorption area as well as area per pit). Conversely, the scramble oligo treatment had no significant effect on the total resorption pit area (Fig. 4, top panel) or area per pit (Fig. 4, bottom panel) in vehicle-, PTH-, or $1,25(OH)_2D_3$-treated osteoclasts. (The PTP-oc sense oligo also had no effect on the total resorption pit area and area per pit [data not shown]). These findings suggest that the PTP-oc antisense oligo (and not the scramble or sense oligo) treatment reduced basal as well as PTH- or $1,25(OH)_2D_3$-induced bone resorption activity of rabbit osteoclasts.

The middle panel of Fig. 4 shows that PTH or $1,25(OH)_2D_3$ treatment had no significant effects on the number of resorption pits and that PTP-oc antisense oligo or scramble oligo pretreatment also did not change significantly the number of resorption pits formed by the treated osteoclasts. Assuming each resorption pit was created by an individual osteoclast, these findings would suggest that PTH and $1,25(OH)_2D_3$ each stimulated osteoclastic resorption without an effect on the life span (i.e., necrosis or apoptosis) of osteoclasts, and that neither the PTP-oc antisense nor the scramble oligo pretreatment had toxic or apoptotic effects on osteoclasts. To further support these contentions, we found that none of the pretreatments (PTP-oc antisense oligo or scramble oligo) and treatments [PTH or $1,25(OH)_2D_3$] changed significantly the number of $TRAP^+$ multinucleated osteoclasts, which remained attached to the dentine slice at the end of the experiment (Table 1), confirming that the PTP-oc antisense oligo is nontoxic to osteoclasts.

To ascertain that the reduction in bone resorption activity of rabbit osteoclasts by the PTP-oc antisense oligo pretreatment was associated with suppression of PTP-oc expression, we next measured the effect of the PTP-oc antisense oligo pretreatment on cellular PTP-oc protein level in osteoclasts treated with or without PTH or $1,25(OH)_2D_3$ in parallel experiments. Figure 5

shows that the PTP-oc antisense oligo pretreatment markedly reduced basal (i.e., vehicle-treated) PTP-oc expression (normalized against actin) to ~40% of that in vehicle-treated control cells ($p < 0.05$, Student's *t*-test), whereas the scramble oligo pretreatment had no significant effect on basal PTP-oc expression. Figure 5 also shows that treatment with 10 nM of PTH or 10 nM of $1,25(OH)_2D_3$ alone (without oligo pretreatment) each increased significantly cellular PTP-oc protein level by ~50% ($p < 0.05$ for each, Student's *t*-test). The scramble oligo pretreatment also did not affect significantly the cellular PTP-oc protein level in PTH- or $1,25(OH)_2D_3$-treated rabbit osteoblasts compared with each respective treated control (pretreated with vehicle only). However, the PTP-oc antisense oligo pretreatment reduced the cellular PTP-oc protein levels (normalized against actin) to 50-60% of respective control in PTH- or $1,25(OH)_2D_3$-treated osteoclasts ($p < 0.05$ for each, Student's *t*-test). Thus, these findings indicate that the PTP-oc antisense oligo pretreatment, and not the scramble oligo pretreatment seemed to block both basal and PTH- or $1,25(OH)_2D_3$-induced PTP-oc expression in isolated rabbit osteoclasts.

Next, we sought to evaluate whether the suppression of osteoclastic resorption mediated by the PTP-oc antisense oligo is associated with an inactivation of the c-*src* in osteoclasts. Because the PTK activity of c-*src* is regulated primarily by the PY-527 tyrosine phosphorylation status in that phosphorylation of tyr-527 in c-*src* led to inactivation of its PTK activity,((17)) we determined if the PTP-oc antisense oligo treatment would increase the overall PY-527 phosphorylation level of c-*src* in treated osteoclasts. Figure 6A shows that the PTP-oc antisense oligo treatment markedly increased the PY527 phosphorylation level of c-*src*. In contrast, the scramble oligo treatment did not significantly affect the c-*src* PY527 phosphorylation level in rabbit osteoclasts. Neither the PTP-oc antisense oligo nor the scramble oligo treatment appeared to affect the total c-*src* protein expression level in osteoclasts (Fig. 6B). Thus, these findings are consistent with the premise that the PTP-oc antisense oligo-mediated inhibition of osteoclastic resorption is associated with an inactivation of the PTK activity of c-*src*.

DISCUSSION

This study shows that a phosphorothioated antisense oligo corresponding to

a unique sequence around the initiation site of the PTP-oc gene was highly effective in suppressing the cellular expression of PTP-oc in isolated rabbit osteoclasts, because the pretreatment with 1 μM of PTP-oc antisense oligo for 3-4 days reduced the cellular PTP-oc protein level to ~20% of the basal level. More importantly, we showed for the first time that suppression of the expression of PTP-oc in rabbit osteoclasts by pretreatment with this PTP-oc antisense oligo was able to reduce markedly basal and PTH-stimulated bone resorption activity as well as $1,25(OH)_2D_3$-stimulated bone resorption activity in the resorption pit formation assay in vitro. Consequently, these observations led us to conclude that this osteoclastic PTP may play an important regulatory function in both basal as well as $1,25(OH)_2D_3$- and PTH-stimulated bone resorption activity of osteoclasts.

We should note that inasmuch as there is evidence that $1,25(OH)_2D_3$ can act directly on osteoclasts to stimulate osteoclastic resorption,((9)) past studies have indicated that the bone resorption stimulatory actions of PTH are mediated through cells of osteoblast lineage to produce a soluble factor, which acts on osteoclast precursors to promote osteoclastic resorption through stimulation of osteoclast differentiation and formation.((18)) Therefore, it is somewhat intriguing that PTH was able to stimulate the bone resorption activity of the isolated rabbit osteoclasts without the coculture with cells of osteoblast lineage in this study. However, we found that the bone resorptive effect of PTH [as well as that of $1,25(OH)_2D_3$] on isolated rabbit osteoclasts in the pit formation assay was identical, regardless of whether or not osteoblast cocultures were included (data not shown). These observations suggest that although the action of PTH to stimulate osteoclast differentiation and formation requires the osteoblast factor, the stimulatory action of PTH on the bone resorption activity of mature rabbit osteoclasts may not require this osteoblastic factor. On the other hand, our osteoclast preparations could contain up to 5% stromal cells. Therefore, we cannot rule out the possibility that PTH might have acted through the contaminating stromal cells to produce a sufficient amount of this osteoblast factor (e.g., receptor activator of $NF\text{-}_\kappa B$ ligand [RANKL]) to stimulate bone resorption activity of the osteoclasts. Regardless of the reasons for the lack of requirement of osteoblast cocultures, this study has shown clearly that PTH and $1,25(OH)_2D_3$ (as well as

prostaglandin E_2) significantly stimulated the bone resorption activity of rabbit osteoclasts in vitro and that their stimulatory actions were abolished completely by the PTP-oc antisense oligo pretreatment.

The functional role of PTP-oc in mediating the basal as well as the 1,25(OH)$_2D_3$- and PTH-stimulated osteoclastic activity has not been determined. Because suppression of PTP-oc expression led to an inhibition of basal and stimulated osteoclastic activity, it follows that the phosphatase activity of PTP-oc is important for the activation of osteoclastic resorption activity. There is strong circumstantial evidence that activation of the PTK activity of c-*src* is essential for the activity of mature osteoclasts.((5-11,19)) In particular, previous studies with specific c-*src* PTK inhibitors have shown that inhibition of c-*src* PTK activity in osteoclasts led to a marked reduction in osteoclast-mediated resorption.((8,20-22)) The c-*src* PTK activity is regulated by its tyrosine phosphorylation status in that the phosphorylation of the tyr-527 residue inactivates (and the dephosphorylation activates) its PTK activity.((17)) In this regard, Chappel and coworkers((9)) have obtained circumstantial evidence that the ability of 1,25(OH)$_2D_3$ to stimulate osteoclast formation and activity may be associated with its ability to stimulate the activity of the c-*src* PTK by dephosphorylating the tyr-527 residue through an increased expression of a PTP in osteoclasts and precursors. Recently, we have also accumulated a large body of preliminary evidence supporting the possibility that the c-*src* PTK is a cellular substrate of PTP-oc in rabbit osteoclasts.((23)) Consequently, it may be speculated that PTP-oc in osteoclasts acts to dephosphorylate PY-527 of c-*src*, leading to the activation of c-*src* PTK activity and the subsequent stimulation of bone resorbing activity of osteoclasts. The finding of this study that the PTP-oc antisense oligo treatment of rabbit osteoclasts reduced the bone resorption activity and at the same time also markedly increased the PY-527 phosphorylation of c-*src* (that presumably would lead to inactivation of the PTK activity of c-*src*) is entirely consistent with this possibility. Therefore, although the evidence supporting this hypothesis is circumstantial, nevertheless, it is an attractive hypothesis, which is worthy of further investigation.

However, we should emphasize that transgenic expression of a K295M "dominant negative" c-*src* mutant in the c-*src*$^{-/-}$ knockout mice could partially

rescue the osteopetrotic phenotype.((12)) Because this K295M dominant negative c-*src* mutant lacks PTK activity, these findings raise an important question as to whether the PTK activity of c-*src* is indeed essential in the osteoclast activation process. On the other hand, the SH2 domain of c-*src* has been proposed to function as an adaptor protein regulating c-*src* trafficking and membrane localization and mediates interactions with downstream signaling proteins and/or recruits key signaling proteins to membrane receptor PTKs.((24-26)) Accordingly, it is conceivable that other functional domains such as the SH-2 domain of c-*src* also could play significant roles in mediating the osteoclast activity. Consistent with this possibility, a recent study shows that bone-targeted inhibitors of Src SH-2 domain blocked osteoclast-mediated resorption.((27)) However, we must not ignore the large volume of compelling evidence implicating an important role of the PTK activity of c-*src* in the activation of osteoclastic resorption.((8,20-22)) Moreover, the PTK activity of c-*src* is likely to play an important role in regulating downstream signaling events, many of which involve PTKs. There is strong evidence that these downstream PTKs, such as Pyk-2 and Cbl, may be involved in the mediation of the bone resorption activity.((10,11)) Thus, although it is likely that other domains (such as SH-2 domain) of c-*src* are important for mediating activity, the PTK activity of c-*src* (or downstream mediators) also may have an important role in mediating and/or enhancing osteoclastic activity. Consequently, we tentatively conclude that the PTK activity of c-*src*, although it may be required, is important for the manifestation of osteoclastic activity. More importantly, our data support our contention that PTP-oc may act to regulate osteoclastic activity through dephosphorylating PY-527 of c-*src* in osteoclasts. Nevertheless, additional work is required to confirm this contention.

In summary, this study provides the first antisense oligo evidence that the expression of PTP-oc and/or its activity is essential for basal as well as 1,25 $(OH)_2D_3$- and PTH-mediated bone resorption activity of isolated rabbit osteoclasts in vitro. If the hypothesis that PTP-oc is vital to the bone resorption activity of osteoclasts is confirmed, PTP-oc may be an attractive target for developing novel, specific, and safer modulators of bone resorption, which can be used as therapeutic agents to treat bone-wasting diseases such as osteoporosis.

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Return to top of article